

EDITORIAL COMMENT

Platelets, the Cardiologist, and Coronary Artery Disease: Moving Beyond Aggregation*

Neal S. Kleiman, MD, FACC

Houston, Texas

Several decades ago, the platelet was viewed as a passive anucleate participant in thrombosis. Its role was thought to be primarily mechanical: to form stable hemostatic plugs that could serve as physical barriers to the flow of blood into unwanted places. For approximately a decade, the primary focus of cardiologists and other practitioners involved in the management of atherosclerosis was directed toward inhibition of the platelet aggregation response. Initially described by Born and Cross (1), this response could be measured easily in either platelet-rich plasma or, with modifications, in whole blood. The description of glycoprotein (GP) IIb/IIIa, the cellular receptor necessary for aggregation (2) and the characterization of a population in whom its

See page 1982

absence (or dysfunction) is reasonably well tolerated (3) led to the development of antibodies, peptides, and wholly synthetic compounds designed specifically to inhibit platelet aggregation. The successful use of these compounds in patients undergoing percutaneous coronary interventions and in those hospitalized with acute coronary syndromes (ACS) (4) further directed the attention of the cardiology community to believe that antiplatelet therapy simply meant prevention of aggregation.

Three surprising findings in the clinical realm led to the re-evaluation of these postulates. First was the finding in more than 19,000 patients enrolled in the Clopidogrel Versus Aspirin in Patients at Risk of Ischaemic Events (CAPRIE) study that a daily dose of clopidogrel, a drug that led to relatively minor decrements in the aggregation response compared with GP IIb/IIIa antagonists, led to a moderate but significant reduction in atherosclerotic morbidity and mortality compared with aspirin (5). When combined with aspirin in patients presenting with ACS in the Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) trial, clopidogrel reduced a composite of cardiovascular death, myocardial infarction, and stroke by 20% compared with aspirin alone (6). A second surprise was

that the combination of the thienopyridine ticlopidine with aspirin was more successful than either aspirin alone or warfarin with aspirin at preventing subacute thrombosis of intracoronary stents (7). The final unexpected finding consisted of serial observations that oral antagonists of GP IIb/IIIa consistently were associated with increases rather than decreases in cardiovascular mortality despite potent and sustained inhibition of platelet aggregation (8). Initially thought to be a consequence of poor pharmacokinetic profiles of the early oral agents, these findings were so consistent from trial to trial that they eventually led to re-examination of the basic paradigm. In fact, these observations were accompanied by findings suggesting that the same agents that prevented platelets from aggregating could simultaneously cause activation of the platelet (9) and increased expression of CD-40 ligand (CD 154) (10), a molecular messenger through which activated platelets initiate an inflammatory response involving endothelial cells and leukocytes.

Taken in aggregate, these findings suggest that the mechanism through which thienopyridines benefit patients with atherosclerosis is likely to extend beyond their relatively mild antiaggregation effects. Thienopyridines, or rather their active metabolites, affect platelet function by inhibiting the action of adenosine diphosphate (ADP) on one of the three platelet purinergic receptors, P2Y₁₂ (11). In doing so, they antagonize the direct effect of ADP released by cells as they are lysed in a developing thrombus, as well as prevent amplification of the initial platelet stimulus that occurs when activated platelets release ADP. Blockade of P2Y₁₂ inhibits expression of the active form of GP IIb/IIIa, thus limiting the formation of large stable platelet aggregates, and prevents platelets from undergoing the shape change reaction, one consequence of which is the expression of P-selectin on the platelet surface (12). In the current issue of the *Journal*, Xiao and Thérout (13) have placed these actions into a clinical perspective and as a result have advanced the field by a giant step. In patients treated with aspirin and clopidogrel for an acute coronary syndrome, they found that a 300 mg loading dose of clopidogrel (in addition to aspirin) produced a modest reduction in turbidimetric platelet aggregation in response to two commonly studied agonists. A more novel finding was an accompanying reduction in platelet-leukocyte complexes and in circulating levels of soluble P-selectin and soluble CD-40 ligand. Treatment with clopidogrel reduced circulating levels of platelet-leukocyte complexes to levels observed in healthy volunteers. In contradistinction, GP IIb/IIIa antagonists, more potent antagonists of platelet aggregation, have been reported to increase, decrease, or not affect platelet-leukocyte complex formation (14–17). Much to their credit, Xiao and Thérout used two different techniques to demonstrate that treatment with clopidogrel interferes with the interaction between platelets and leukocytes. In many previous studies, distinction between antiplatelet effects and

*Editorials published in the *Journal of the American College of Cardiology* reflect the views of the authors and do not necessarily represent the views of JACC or the American College of Cardiology.

From the Cardiac Catheterization Laboratory, Baylor College of Medicine, Methodist DeBakey Heart Center, Houston, Texas.

prevention of platelet-leukocyte adhesion (i.e., complex formation) has been obscured by flow cytometric techniques that count platelet-leukocyte complexes by measuring the fluorescence of platelets bound to leukocytes. Because activated platelets bind to other platelets as well as to leukocytes, merely counting platelet numbers within a leukocyte gate cannot distinguish whether the number of platelets per platelet-leukocyte complex is diminished or whether the absolute number of complexes is decreased. These authors have measured both entities and demonstrated that the numbers of complexes as well as the platelet density per complex are decreased.

This finding is of importance because the details of interactions between platelets and leukocytes are only beginning to be appreciated. As leukocytes migrate to sites of vascular injury, they are able to roll along the endothelial surface without becoming activated. There, they are exposed to a variety of activating factors, including activated endothelial cells and activated platelets adherent to the subendothelium. Both of the latter cell types express P-selectin on their surfaces. The presentation of activated platelets to the leukocyte is believed to occur as a multi-step process beginning with the attachment of platelet P-selectin to leukocyte PSGL-1, a receptor constitutively expressed on leukocytes. Blocking antibodies directed at either P-selectin or PSGL-1 can prevent this interaction or even disaggregate platelet-leukocyte complexes (14). Subsequent activation of the leukocyte leads to the formation of much firmer bonds through integrins, most likely CD 11b/CD18 and its counter-receptor GPIIb. This process has two important implications for those interested in the mechanisms of thrombosis and inflammation. First, because the expression of P-selectin on the platelet surface requires that the platelet itself be activated, platelet-leukocyte complexes have long been regarded as markers of platelet activation. Second, it is now becoming clear that these interactions have functional implications. Activated platelets are known to stimulate proinflammatory and prothrombotic responses in leukocytes and endothelial cells (18). Most of the steps involved have been worked out in monocytes and monocyte-derived cell lines. Ligation of PSGL-1 by P-selectin results in several important changes in the monocyte, including conversion of the cell membrane to a prothrombotic surface capable of binding procoagulant proteins, surface expression of tissue factor that can initiate the coagulation cascade, and permitting the activated monocyte to diapedese across an endothelial surface into the subendothelium. It also appears that trafficking in tissue factor occurs between platelets and monocytes. Blockade of P2Y₁₂ markedly reduces tissue factor expression on monocytes in a whole blood model (19). The current findings thus reveal that in the patient group shown to benefit from treatment with clopidogrel, blockade of platelet P2Y₁₂ can prevent platelet contact with monocytes and neutrophils. The next step in understanding these mechanisms will be to determine whether interdiction of this pathway has functional consequences (i.e., whether it

inhibits leukocyte migration into the diseased subintima and whether it prevents leukocytes from exerting proinflammatory and other prothrombotic effects). Such a finding would provide considerable support for the hypothesis that an indirect anti-inflammatory is part of the mechanism of the long-term benefit of thienopyridines observed after percutaneous interventions and in patients with ACS.

Several other questions also need to be answered. Recent data indicate that at any given time after administering clopidogrel, the degree to which platelet aggregation and expression of P-selectin are inhibited is subject to wide variation. This phenomenon, termed “resistance,” may be due to variations in P2Y₁₂ receptor expression, intracellular signaling mechanisms, or the production of the active metabolite (20). Polymorphisms of P2Y₁₂ receptor with functional consequences *in vitro* also have been reported (21). It is likely but not established that the anti-inflammatory effect of clopidogrel also is subject to such variability. Questions also remain about the appropriate loading dose of clopidogrel. In the initial studies, patients were begun on 75 mg during the first day of therapy. Subsequent data indicated that a loading dose of 300 mg could achieve more rapid inhibition of platelet aggregation, and this dose was used in the CURE trial (22). More recent data indicate that a loading dose of 600 mg is able to inhibit platelet aggregation even more rapidly (23); this dose has been used in a more recent trial in patients undergoing percutaneous coronary intervention. Will the 600-mg dose have more potent or more reliable effects on platelet-leukocyte complexes and their functional sequelae? Finally, will concomitant blockade of the other human platelet purinergic receptor, P2Y₁, enhance this effect? Although these questions are not correctly answered, it is important to recognize that an important step has been made here that should help guide future research directed at platelet purinergic receptors and their antagonism.

Reprint requests and correspondence: Dr. Neal S. Kleiman, 6565 Fannin, MS F1090, Houston, Texas 77030. E-mail: nkleiman@bcm.tmc.edu.

REFERENCES

1. Born GV, Cross MJ. The aggregation of blood platelets. *J Physiol (Lond)* 1963;168:178–95.
2. Marguerie GA, Plow EF, Edgington TS. Human platelets possess an inducible and saturable receptor specific for fibrinogen. *J Biol Chem* 1979;254:5357–63.
3. Collier BS, Seligsohn U, Peretz H, Newman PJ. Glanzmann thrombasthenia: new insights from an historical perspective. *Semin Hematol* 1994;31:301–11.
4. Kong DF, Califf RM. Glycoprotein IIb/IIIa receptor antagonists in non-ST elevation acute coronary syndromes and percutaneous revascularisation: a review of trial reports (review). *Drugs* 1999;58:609–20.
5. CAPRIE Steering Committee. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). *Lancet* 1996;348:1329–39.
6. Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK, for the Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial Investigators. Effects of clopidogrel in addition to aspirin in patients

- with acute coronary syndromes without ST-segment elevation. *N Engl J Med* 2001;345:494–502.
7. Leon MB, Baim DS, Popma JJ, et al. A clinical trial comparing three antithrombotic-drug regimens after coronary-artery stenting. Stent Anticoagulation Restenosis Study Investigators. *N Engl J Med* 1998; 339:1665–71.
 8. Chew DP, Bhatt DL, Sapp S, Topol EJ. Increased mortality with oral platelet glycoprotein IIb/IIIa antagonists: a meta-analysis of phase III multicenter randomized trials. *Circulation* 2001;103:201–6.
 9. Holmes MB, Sobel BE, Cannon CP, Schneider DJ. Increased platelet reactivity in patients given orbofiban after an acute coronary syndrome: an OPUS-TIMI 16 substudy. Orbofiban in Patients with Unstable coronary syndromes. Thrombolysis In Myocardial Infarction. *Am J Cardiol* 2000;85:491–3, A10.
 10. Nannizzi-Alaimo L, Alves VL, Phillips DR. Inhibitory effects of glycoprotein IIb/IIIa antagonists and aspirin on the release of soluble CD40 ligand during platelet stimulation. *Circulation* 2003;107: 1123–8.
 11. Hollopeter G, Jantzen H-M, Vincent D, et al. Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature* 2001;409:202–6.
 12. Cattaneo M, Gachet C. ADP receptors and clinical bleeding disorders. *Arterioscler Thromb Vasc Biol* 1999;19:2281–5.
 13. Xiao Z, Théroux P. Clopidogrel inhibits platelet-leukocyte interactions and thrombin receptor agonist peptide-induced platelet activation in patients with an acute coronary syndrome. *J Am Coll Cardiol* 2004;43:1982–8.
 14. Fernandes LS, Conde I, Smith CW, et al. Platelet-monocyte complex formation: effect of blocking PSGL-1, alone and in combination with alpha IIb-beta 3 and alpha M-beta 2, in coronary stenting. *Thromb Res* 2003;111:171–7.
 15. Mickelson JK, Ali MN, Kleiman NS, et al. Chimeric 7E3 Fab (ReoPro) decreases detectable CD11b on neutrophils from patients undergoing coronary angioplasty. *J Am Coll Cardiol* 1999;33:97–106.
 16. Zhao L, Bath PM, Fox S, et al. The effects of GPIIb-IIIa antagonists and a combination of three other antiplatelet agents on platelet-leukocyte interactions. *Curr Med Res Opin* 2003;19:178–86.
 17. Caron A, Theoret JF, Mousa SA, Merhi Y. Anti-platelet effects of GPIIb/IIIa and P-selectin antagonism, platelet activation, and binding to neutrophils. *J Cardiovasc Pharmacol* 2002;40:296–306.
 18. Lindmark E, Tenno T, Siegbahn A. Role of platelet P-selectin and CD40 ligand in the induction of monocytic tissue factor expression. *Arterioscler Thromb Vasc Biol* 2000;20:2322–8.
 19. Leon C, Alex M, Klocke A, et al. Platelet ADP receptors contribute to the initiation of intravascular coagulation. *Blood* 2003;2003–5.
 20. Gurbel PA, Bliden KP, Hiatt BL, O'Connor CM. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. *Circulation* 2003;107:2908.
 21. Fontana P, Dupont A, Gandrille S, et al. Adenosine diphosphate-induced platelet aggregation is associated with P2Y12 gene sequence variations in healthy subjects. *Circulation* 2003;108:989–95.
 22. Cadroy Y, Bossavy JP, Thalameas C, Sagnard L, Sakariassen K, Boneu B. Early potent antithrombotic effect with combined aspirin and a loading dose of clopidogrel on experimental arterial thrombogenesis in humans. *Circulation* 2000;101:2823–8.
 23. Muller I, Seyfarth M, Rudiger S, et al. Effect of a high loading dose of clopidogrel on platelet function in patients undergoing coronary stent placement. *Heart* 2001;85:92–3.